

**AMENDMENTS TO THE SPECIFICATION**

Please replace the paragraphs from page 8, lines 21-24 with the following:

Figure 1 is a list of the top 100 biomarkers identified with an IMAC3Cu **ProteinChip®** **PROTEINCHIP (biochip with functionalized, cross-linked polymer)** array format, ranked according to p value in a student t-test.

Figure 2 is a list of the top 100 biomarkers identified with a WCX **ProteinChip®** **PROTEINCHIP (biochip with functionalized, cross-linked polymer)** array format, ranked according to p value in a student t-test.

Please replace the paragraph beginning at page 10, line 12 with the following:

All of the biomarkers are characterized by molecular weight, and two lists of biomarkers within the present invention are provided in Figures 1 and 2. These figures list the top 100 biomarkers, as determined statistically by p value, that are identified by Cu(II)IMAC3 and WCX2 **ProteinChip®** **PROTEINCHIP (biochip with functionalized, cross-linked polymer)** array protocols described herein, respectively. In each figure, the number in the first column is the biomarker identifier. Thus, the first row in Figure 1 relates to biomarker I-M1, the second row relates to biomarker I-M2, and so forth (“I-M” denoting biomarkers identified with the IMAC chip). Similarly, the first row in Figure 2 relates to biomarker W-M1 and the second row relates to biomarker W-M2 (“W-M” denoting biomarkers identified with the WCX2 chip). The number in the second column of the figures is the apparent molecular weight of the biomarker in daltons, as determined by gas phase ion spectrometry. The letter in the final column of the figures denotes the fraction in which the biomarker elutes in the protocol described herein; that is, biomarkers with an “A” elute in first fraction, biomarkers with a “B” elute in the second fraction, and so forth. The fraction in which the biomarker elutes correlates with its pI, which biomarkers eluting at higher pH having a higher pI, and biomarkers eluting at lower pH having a lower pI.

Please replace the paragraph beginning at page 12, line 12 with the following:

Each fraction was diluted and applied to a **ProteinChip® PROTEINCHIP (biochip with functionalized, cross-linked polymer)** array, either an Cu(II) (IMAC3) or WCX2 chip array. Both of these chip arrays are produced by Ciphergen Biosystems, Inc. (Fremont, CA).

Please replace the paragraph beginning at page 13, line 11 with the following:

In the mass spectrometer, retained polypeptides were eluted from the chip array by laser desorption and ionization in a **ProteinChip® Reader PROTEINCHIP Reader (Vermillion, Inc., Fremont, CA)**, which is integrated with **ProteinChip® Software PROTEINCHIP Software (Vermillion, Inc., Fremont, CA)** and a personal computer to analyze proteins captured on chip arrays. The ion optic and laser optic technologies in the **ProteinChip® Reader PROTEINCHIP Reader (Vermillion, Inc., Fremont, CA)** detects proteins ranging from small peptides of less than 1000 Da up to proteins of 300 kilodaltons or more, and calculates the mass based on time-of-flight. Ionized polypeptides were detected and their mass accurately determined by this Time-of-Flight (TOF) Mass Spectrometry.

Please replace the paragraph beginning at page 14, line 24 with the following:

A total of 2384 proteomic features were found among the serum samples: 1087 by using the IMAC3 copper **ProteinChip® PROTEINCHIP (biochip with functionalized, cross-linked polymer)** Array and 1297 by using the WCX2 **ProteinChip® PROTEINCHIP (biochip with functionalized, cross-linked polymer)** Array. SAM for protein filtering was used to search for the serum proteins/polypeptides significantly different between the HCC and CLD cases. By setting the median value of false significant number  $< 0.000005$ , 79 proteomic features were identified to be significantly higher in the HCC patient sera, and 160 proteomic features were significantly lower. Thus, 239 potential serological markers for the identification of HCC were found, in total. Table 1 lists five each of the most significantly higher and lower proteomic features.

Please replace the paragraph beginning at page 25, line 5 with the following:

Illustrative of Ciphergen **ProteinChip® PROTEINCHIP (biochip with functionalized, cross-linked polymer)** arrays are biochips H4, SAX-2, WCX-2, and IMAC-3, which include a functionalized, cross-linked polymer in the form of a hydrogel, physically attached to the surface of the biochip or covalently attached through a silane to the surface of the biochip. The H4 biochip has isopropyl functionalities for hydrophobic binding. The SAX-2 biochip has quaternary ammonium functionalities for anion exchange. The WCX-2 biochip has carboxylate functionalities for cation exchange. The IMAC-3 biochip has nitriloacetic acid functionalities that adsorb transition metal ions, such as Cu<sup>++</sup> and Ni<sup>++</sup>, by chelation. These immobilized metal ions, in turn, allow for adsorption of biomarkers by coordinate covalent bonding. Thus, Ciphergen's IMAC **ProteinChip® PROTEINCHIP (biochip with functionalized, cross-linked polymer)** arrays are sold with reactive moieties that become adsorbent upon the addition by the user of a metal solution.

Please replace the paragraph beginning at page 28, line 26 with the following:

In a preferred embodiment, the detection of biomarkers for diagnosis of hepatocellular carcinoma in a subject entails contacting a sample from a subject or patient, preferably a serum sample, with a substrate having an adsorbent thereon under conditions that allow binding between the biomarker and the adsorbent, and then detecting the biomarker bound to the adsorbent by gas phase ion spectrometry, preferably by Surface Enhanced Laser Desorption/Ionization (SELDI) mass spectrometry. The biomarkers are ionized by an ionization source such as a laser. The generated ions are collected by an ion optic assembly and accelerated toward an ion detector. Ions that strike the detector generate an electric potential that is digitized by a high speed time-array recording device that digitally captures the analog signal. Ciphergen's **ProteinChip® PROTEINCHIP** system (**Fremont, CA**) employs an analog-to-digital converter (ADC) to accomplish this. The ADC integrates detector output at regularly spaced time intervals into time-dependent bins. The time intervals typically are one to four nanoseconds long. Furthermore, the time-of-flight spectrum ultimately analyzed typically does not represent the signal from a single pulse of ionizing energy against a sample, but rather the sum of signals from a number of pulses. This reduces noise and increases dynamic range. This time-of-flight data is then subject to data processing. In Ciphergen's **ProteinChip® PROTEINCHIP** software (**Fremont, CA**), data

processing typically includes TOF-to-M/Z transformation, baseline subtraction, high frequency noise filtering. Thus, both the quantity and mass of the biomarker can be determined.

Please replace paragraphs from page 32, line 16 to page 33, line 19 with the following:

**ProteinChip® PROTEINCHIP (biochip with functionalized, cross-linked polymer)** Arrays were set up in 96-well bioprocessors. Buffer delivery and sample incubation were performed on a Biomek 2000 Automation Workstation. Each serum fraction was analyzed on IMAC3 (loaded with copper) and WCX2 **ProteinChip® PROTEINCHIP (biochip with functionalized, cross-linked polymer)** Arrays in duplicates. The different **ProteinChip PROTEINCHIP (biochip with functionalized, cross-linked polymer)** surfaces (2<sup>nd</sup> dimension) helped to identify very low abundance proteins. The IMAC3 copper and WCX2 **ProteinChip PROTEINCHIP (biochip with functionalized, cross-linked polymer)** surfaces preferentially retain different groups of proteins according to their physiochemical properties.

The IMAC3 copper and WCX2 arrays (Ciphergen Biosystems Inc, Fremont, CA) were equilibrated two times with 150µL of binding buffer (100mM sodium phosphate + 0.5M NaCl pH7 for IMAC3, 100mM sodium acetate pH4 for WCX2). Each serum fraction was diluted in the corresponding binding buffer (1/5 dilution for IMAC3 and 1/10 dilution for WCX2) and 100µL was applied to each **ProteinChip® PROTEINCHIP (biochip with functionalized, cross-linked polymer)** array. Incubation was performed on a platform shaker at room temperature for 30 minutes. Each array was washed three times with 150µL of corresponding binding buffer and rinsed two times with water. **ProteinChip® PROTEINCHIP (biochip with functionalized, cross-linked polymer)** arrays were air-dried. Sinapinic acid matrix (prepared in 50% acetonitrile, 0.5% trifluoroacetic acid) was applied to each array.

**ProteinChip® PROTEINCHIP (biochip with functionalized, cross-linked polymer)** arrays were read on a **ProteinChip® PROTEINCHIP** PBSII Reader (Ciphergen Biosystems Inc.) to measure the masses and intensities of the protein peaks (Ciphergen). A total of 253 laser shots were averaged for each array. The mass spectrometric analysis (3<sup>rd</sup> dimension) with the **ProteinChip PROTEINCHIP** PBS II reader can be regarded as a higher resolution substitution of the 2<sup>nd</sup> dimensional separation, SDS-PAGE, in the 2D PAGE technology. Both technologies separate the proteins on the basis of their molecular weights. 235 laser shots were averaged for each array with mass ranging from 0 to 200 kDa. All the mass spectra were normalized to have the same total ion current. The CVs of the peak intensities were less than 15% (manufacturer

Serial No. 10/508,781

Atty. Dkt. No. 035394-0265

information). Common protein peaks were picked by the **Biomarker Wizard™ BIOMARKER WIZARD** function of the **ProteinChip PROTEINCHIP** Software (Ciphergen).